

Fig. 1a: Nuc template/ primer systems:

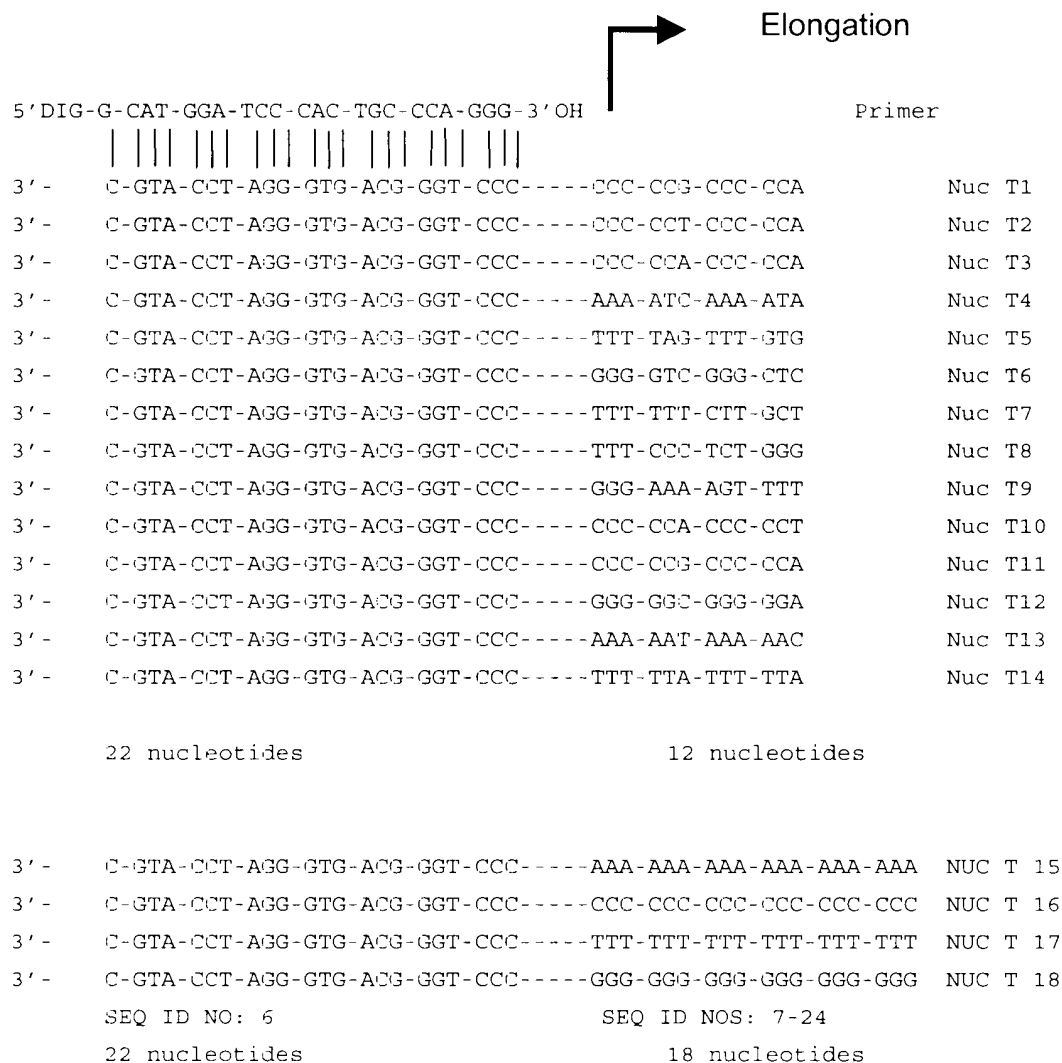


Fig.1b: Cassettes 1-10 template/ primer systems:

GTAAAGATTGCCTCTAGCGG	CGTA	CAS 1
GTAAAGATTGCCTCTAGCGG	CGTA-GTAC	CAS 2
GTAAAGATTGCCTCTAGCGG	CGTA-GTAC-TAGC	CAS 3
GTAAAGATTGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG	CAS 4
GTAAAGATTGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG	CAS 5
GTAAAGATTGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT	CAS 6
GTAAAGATTGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT-TGCA	CAS 7
GTAAAGATTGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT-TGCA-TGAC	CAS 8
GTAAAGATTGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT-TGCA-TGAC-CAGT	CAS 9
GTAAAGATTGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT-TGCA-TGAC-CAGT-GATC	CAS 10

SEQ ID NO: 25

GTAAAGATTGCCTCTAGCGG



AATTCTAAGCGGAGATCGCC-3'

Primer-5' DIG

SEQ ID. NO: 26



Elongation

Fig. 2

1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	Lane
2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	number
Taq-Pol		Tgo exo <sup>-</sup> polymerase															Polymerase unit/ reaction Template (T)
0,1 u		0,1u		1 u		0,1u		1u		0,1u		1 u	1 u				
T 17	T 16	T 17	T 16	T 17	T 16	T 8				T 3							
natural dNTP's		AA BB		AA BB		AAA AAA BB BB C C		AAAAAA BB BB C C		B B D		BB D	BBBB EEFF		Derivative		



← Primer

Fig. 3

1	3	5	7	9	11	13	15	17	19	21	23	25	27	29
2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Controls <i>Taq</i> pol			<i>Tgo</i> exo <sup>-</sup> polymerase 0.1 unit/reaction				<i>Tgo</i> exo <sup>-</sup> polymerase 1 unit/reaction							
			T4	T15	T4	T15	T4	T15	T4	T15	T4	T15	T4	T15
Reaction time: 30 min											60 min			
regular dNTP's			Rho-green- dUTP		Rho- green- X-dUTP		Rho- green- dUTP		Rho- green- X-dUTP		Rho- green- dUTP		Rho- green- X- dUTP	

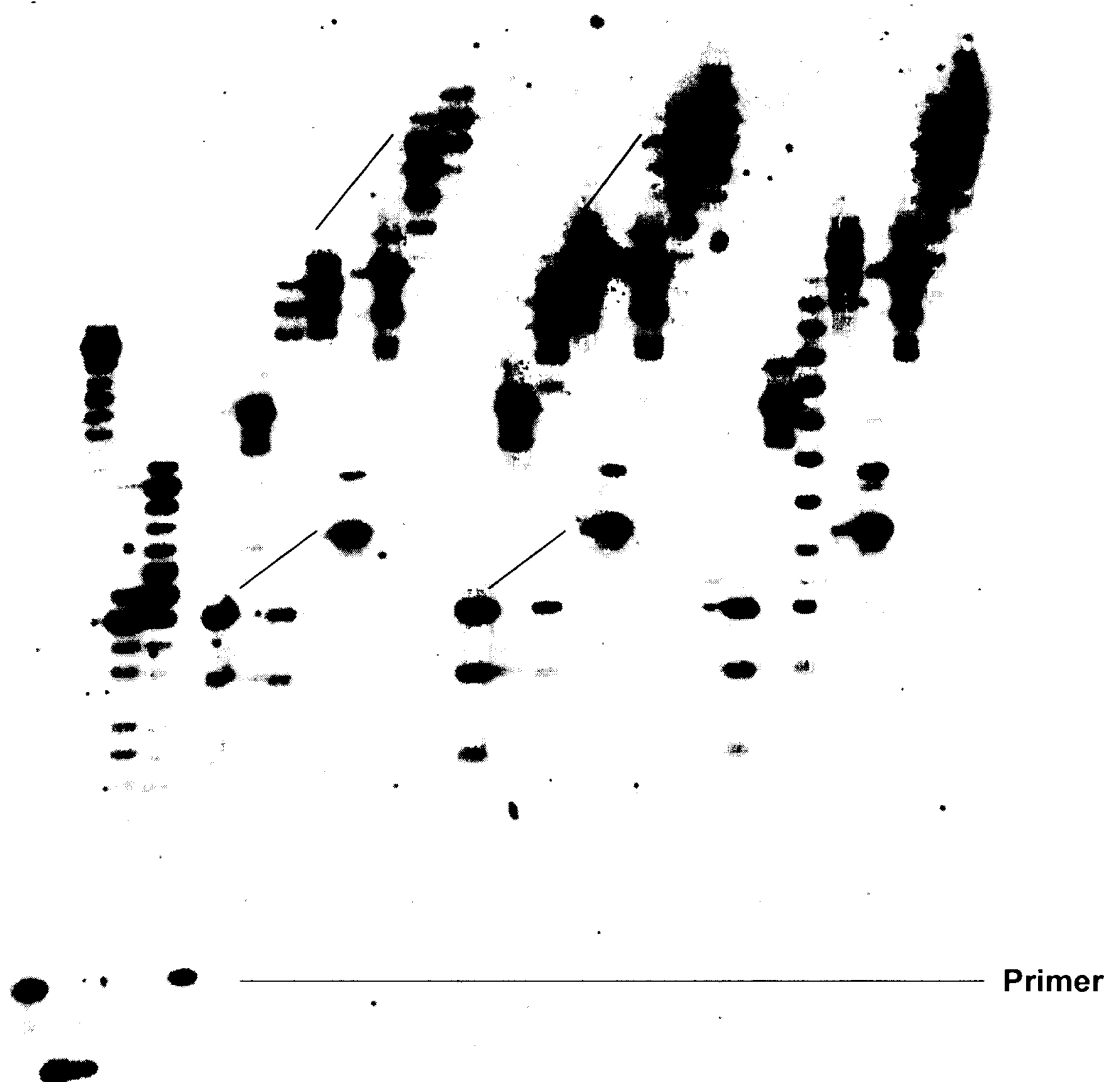
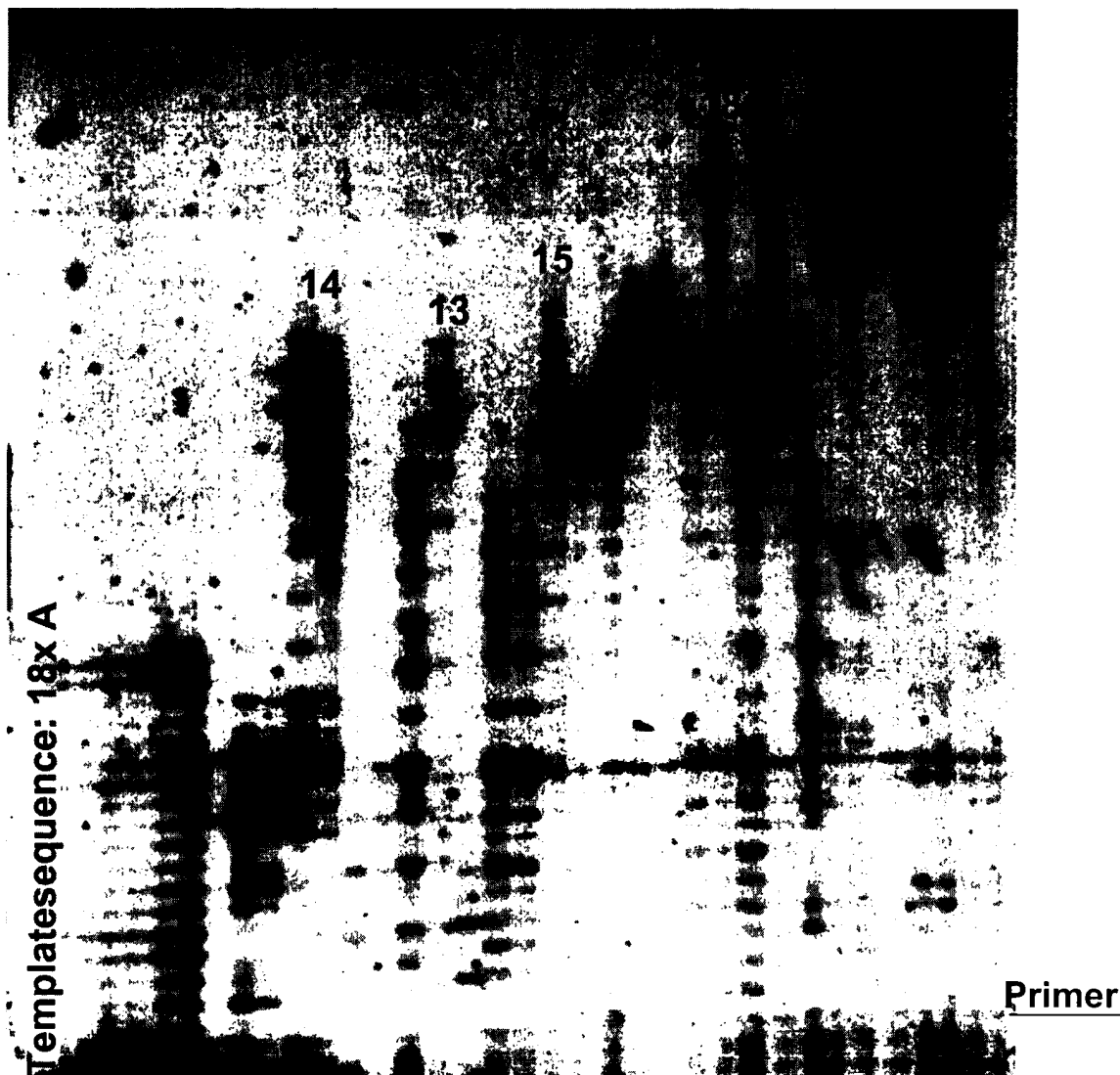


Fig. 4

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33  
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34



Controls	TMR	Cy5	Fluos	AMCA	Fluos	Biotin	Dig
Taq	Vent exo -		Chy-Klen.			Tgo exo -	

Fig. 5

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35  
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34

Primer



Control *Taq*

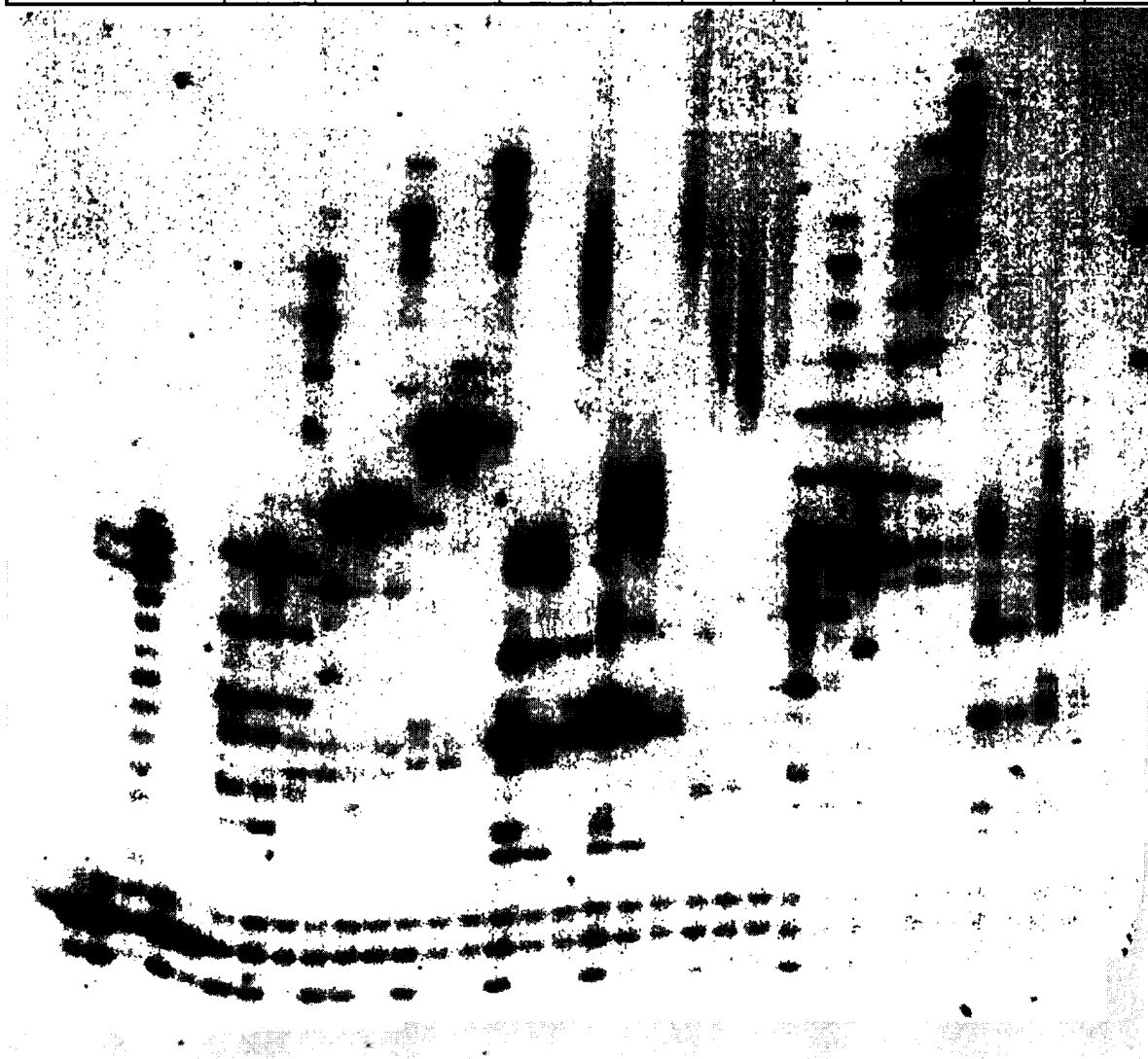
*Taq* polymerase

*Chy*-Klenow-fragment

Fig. 6

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35  
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34

Taq-pol control		Tgo exo <sup>-</sup> pol										
Template T4							Template T15					
natural dNTP's	Cy5-17-dUTP	Cy5-24-dUTP	Cy5-38-dUTP	MR121-8-dUTP	MR121-13-dUTP	MR121-24-dUTP	Cy5-17-dUTP	Cy5-24-dUTP	Cy5-38-dUTP	MR121-8-dUTP	MR121-13-dUTP	MR121-24-dUTP



Primer

Fig. 6a

10/019850

10/019850

11 13 15 17  
1 2 3 4 5 6 7 8 9 10 12 14 16 18

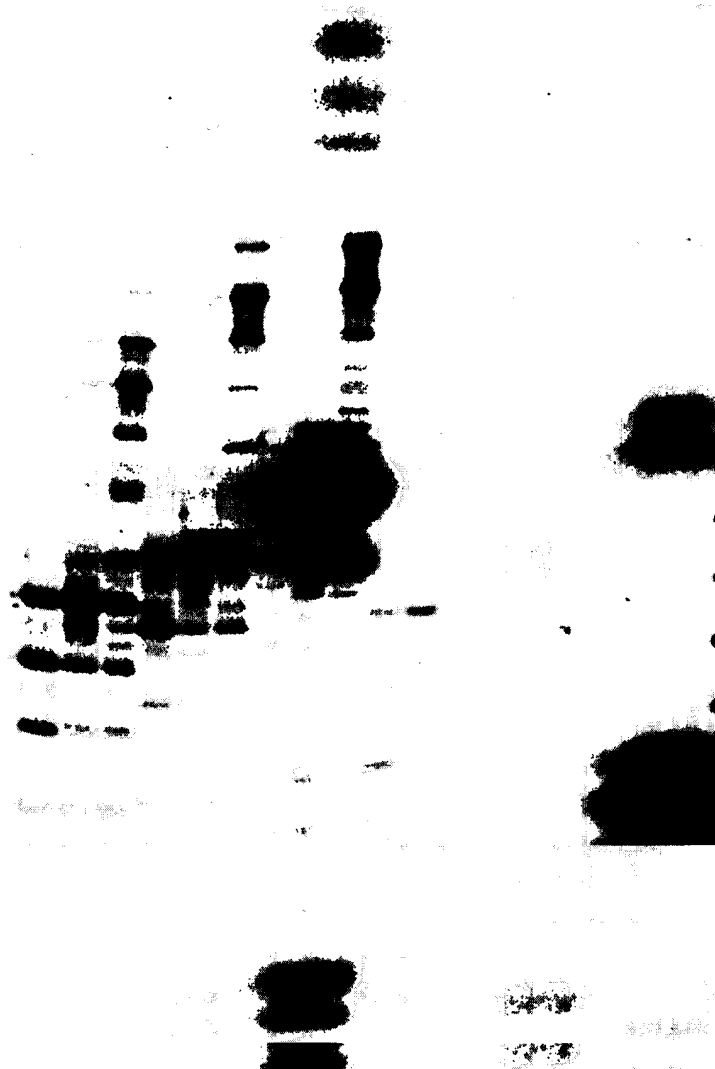


Fig. 7

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35  
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

<i>Taq-pol</i>	<i>Vent</i>	<i>Tgo</i>	<i>Vent</i>	<i>Tgo</i>	<i>Vent</i>	<i>Tgo</i>	<i>Vent</i>
0,1 unit	0,1 unit				0,5 unit		1u
cass - 1 1 5 10 -	cass 1		cass 5		cass 5	cass 10	cass 10
control reactions natural dNTP's	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA
	BBB	BBB	BBB	BBB	BBB	BBB	BBB
	CC	CC	CC	CC	CC	CC	CC
	D	D	D	D	D	D	D



Fig. 8

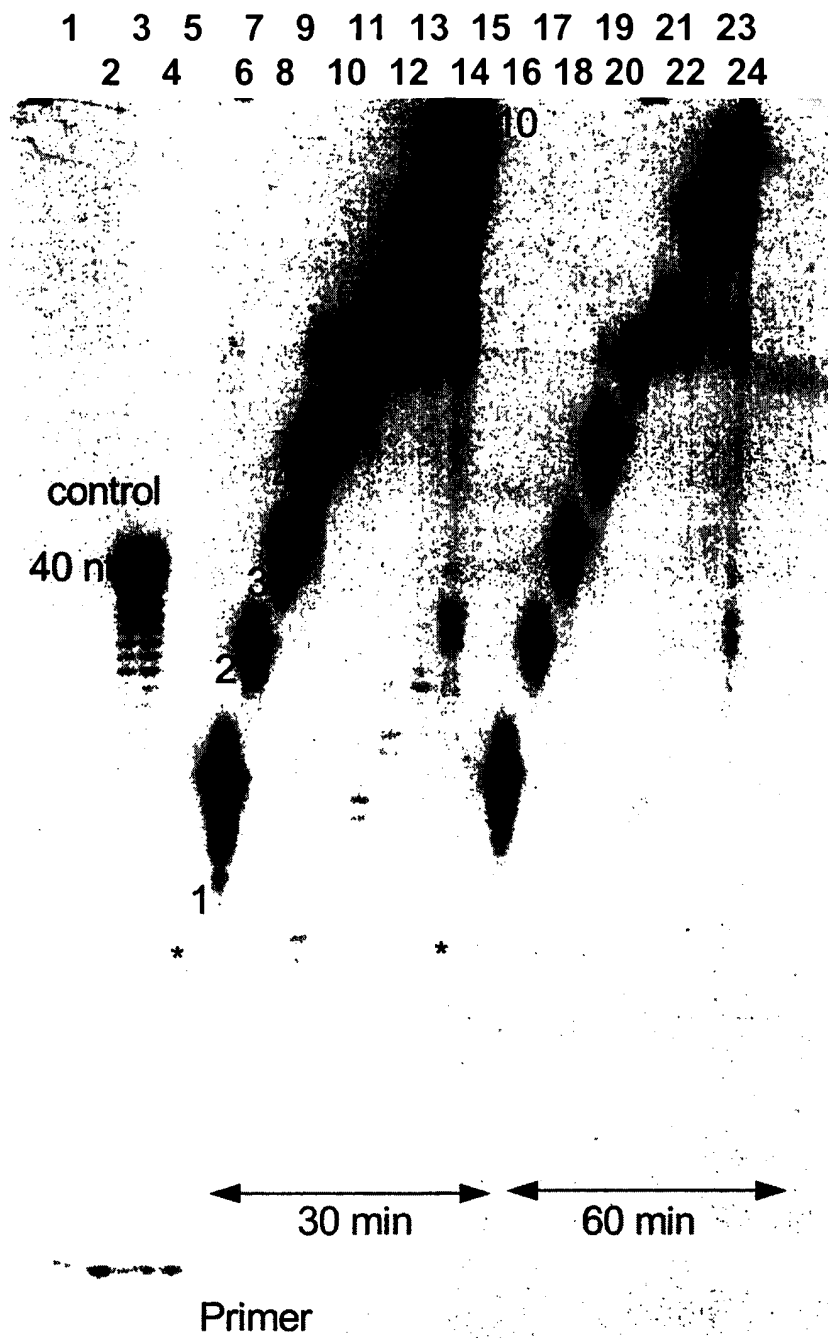
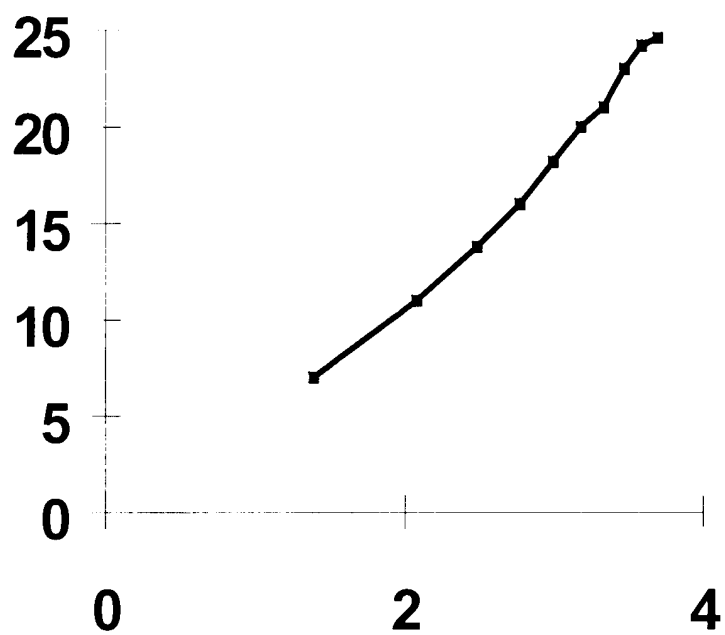


Fig. 8a



Ln of number of incorporated nucleotides

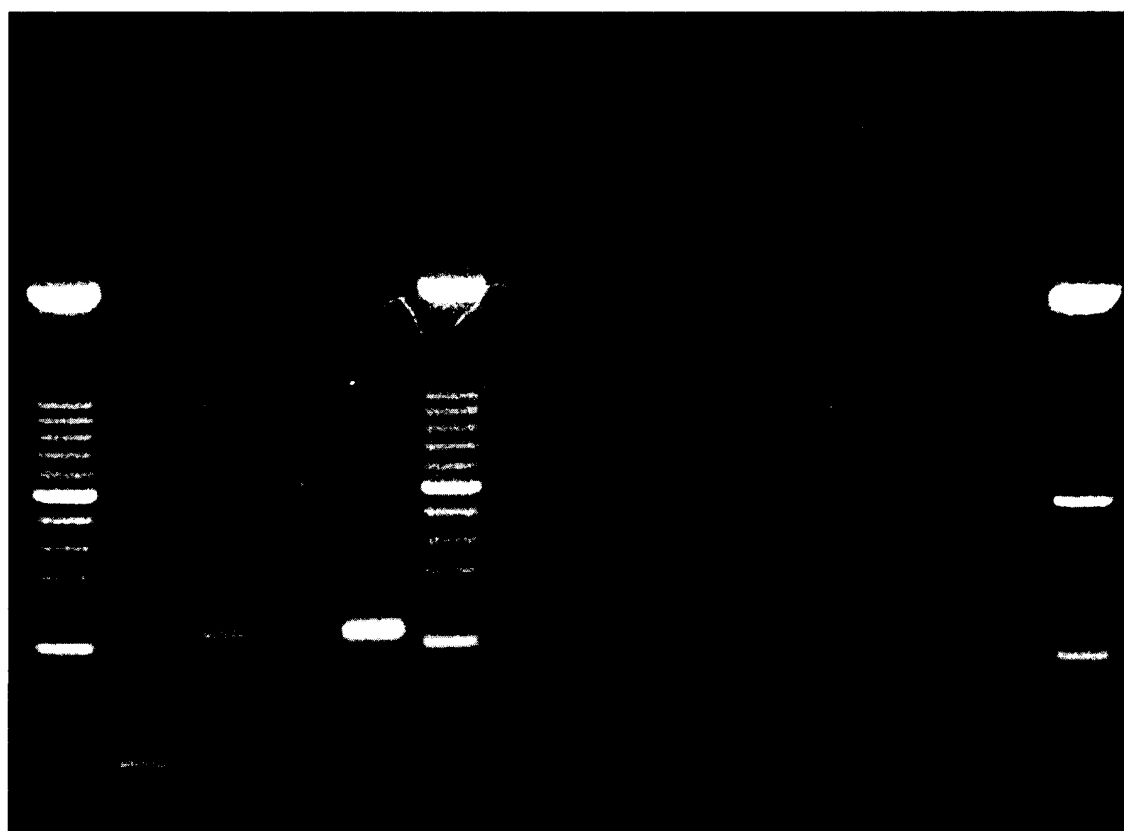
Fig. 9

10/019850

Template concentration



M A B C D M K E F G H I J M



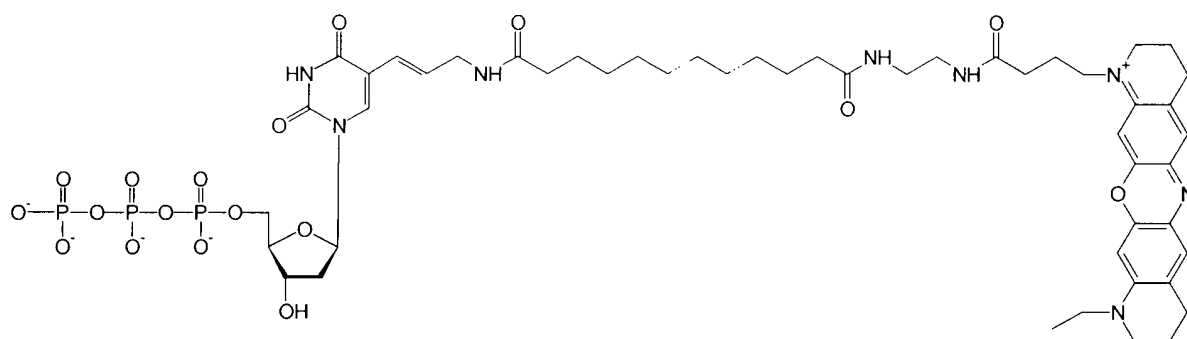
— 1000 bp

— 500 bp

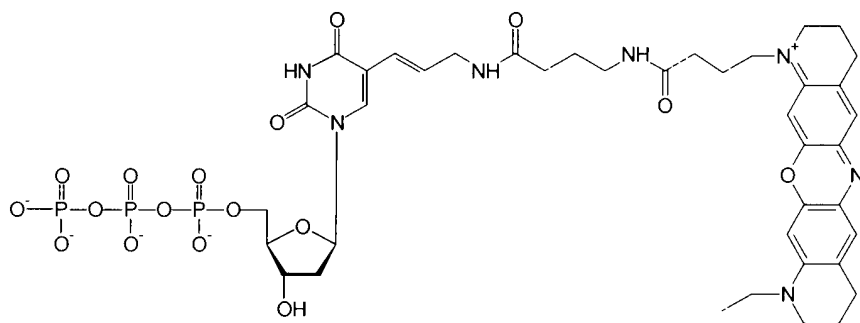
Fig. 10

CONFIDENTIAL  
10-019896

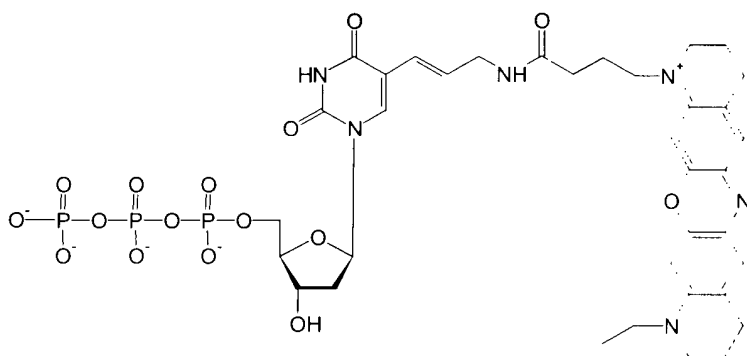
Formula of derivatives used:



MR-121-24-dUTP

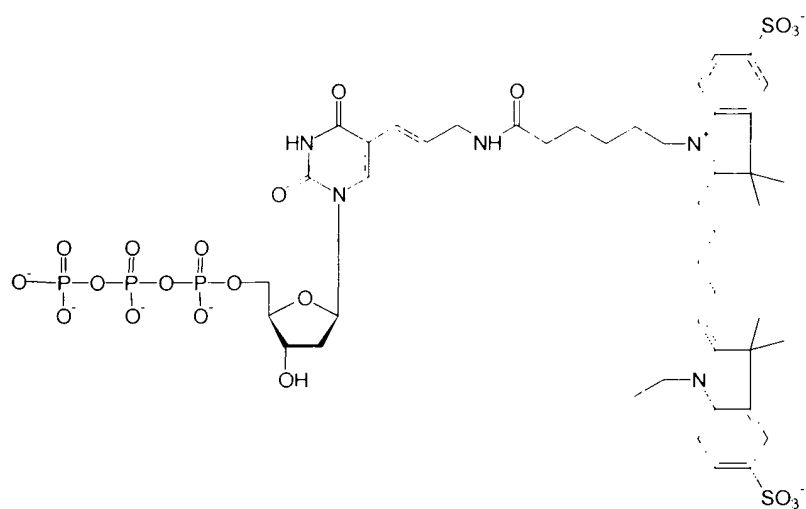


MR-121-13-dUTP

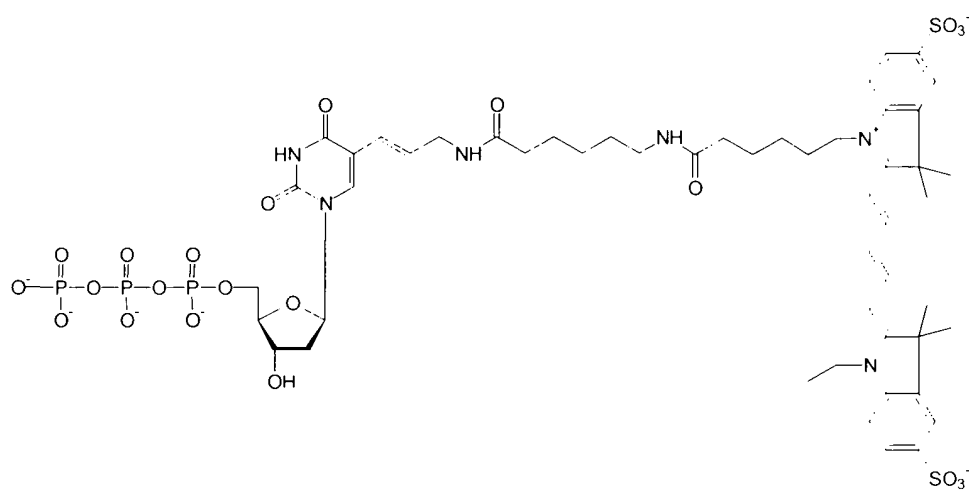


MR-121-8-dUTP

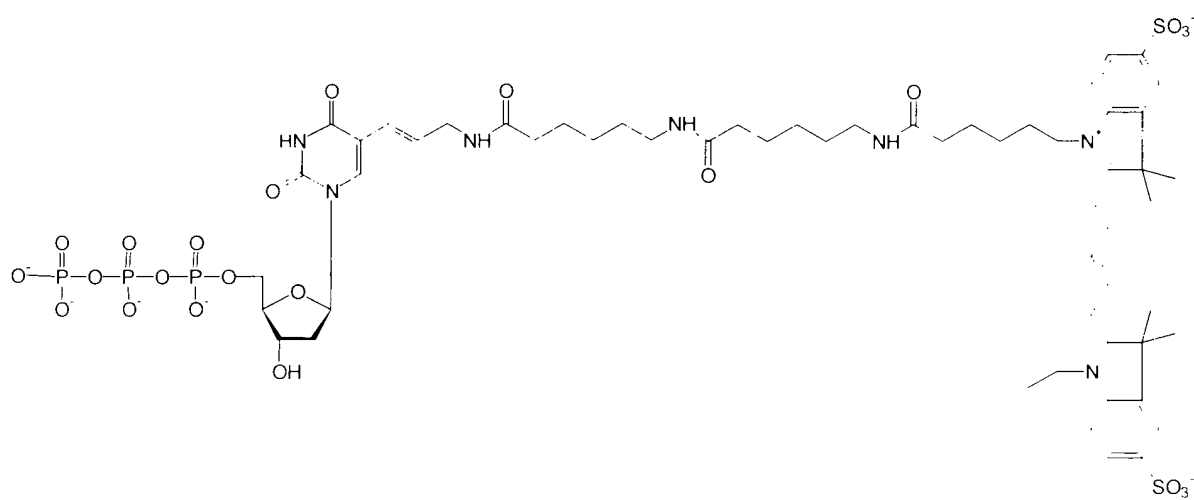
Fig. 11



Cy5-10-dUTP



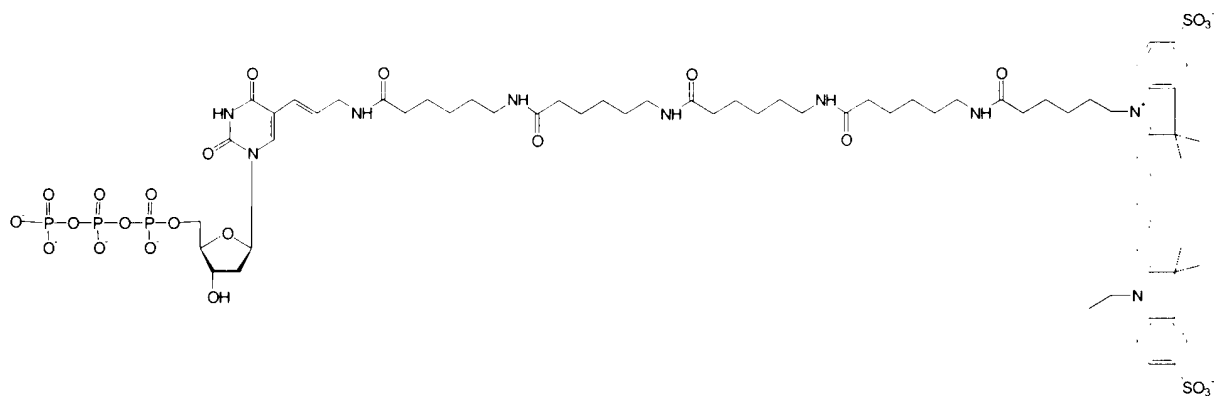
Cy5-17-dUTP



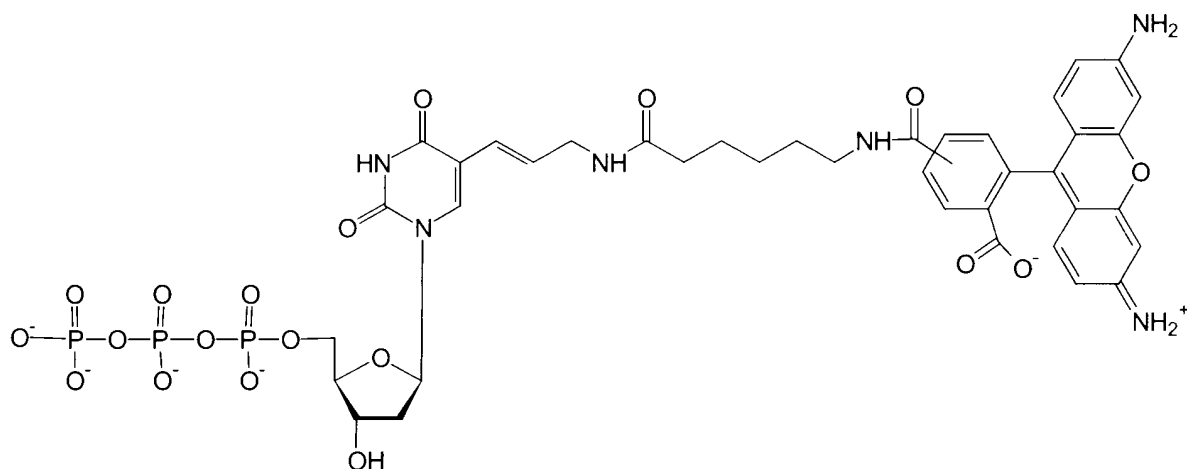
Cy5-24-dUTP

Fig. 12

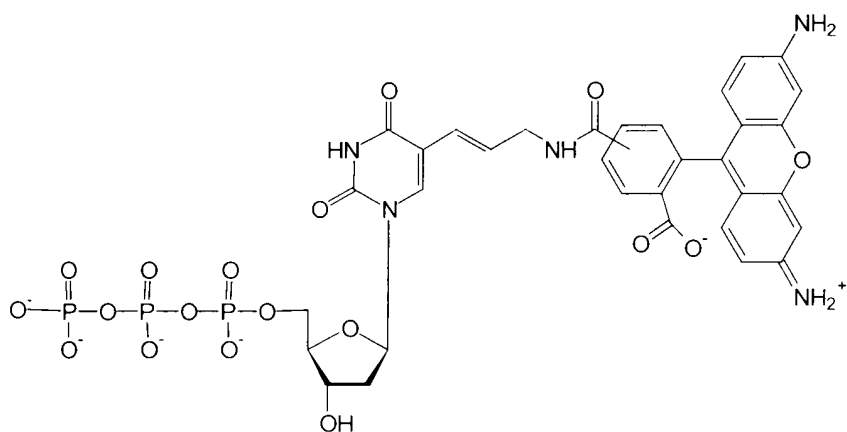
10/019830



Cy5-38-dUTP



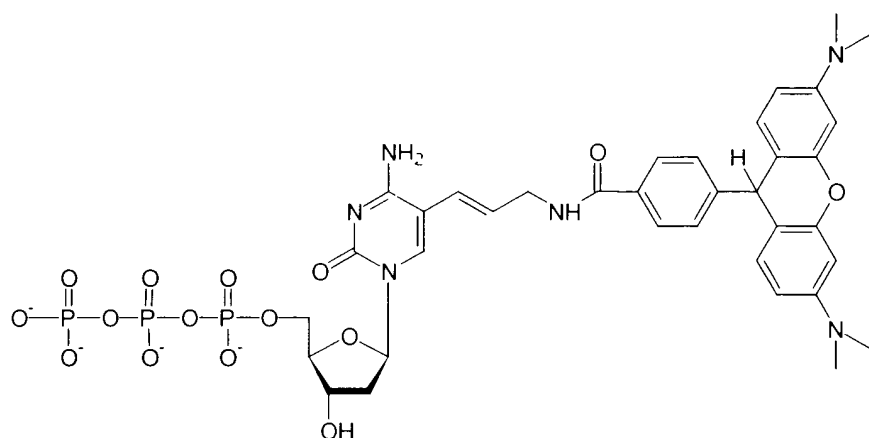
Rhodamin-green-x-dUTP



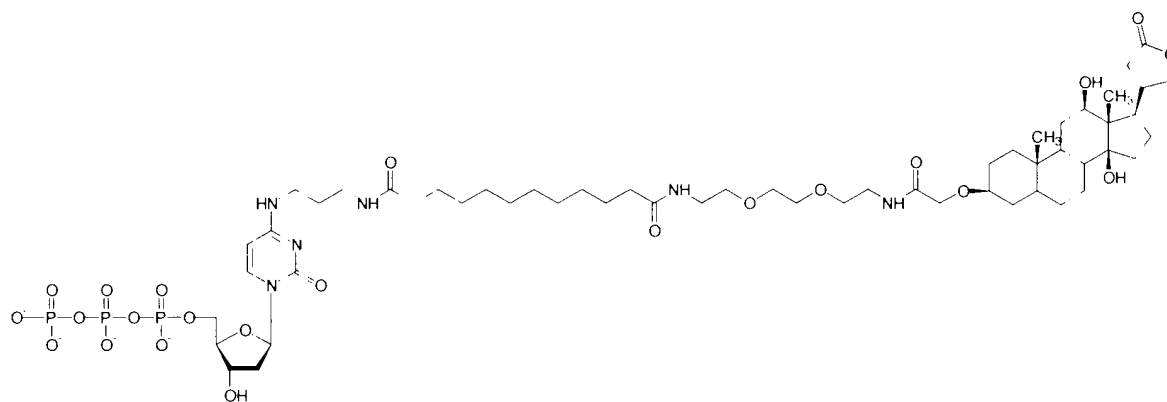
Rhodamin-green-dUTP

Fig. 13

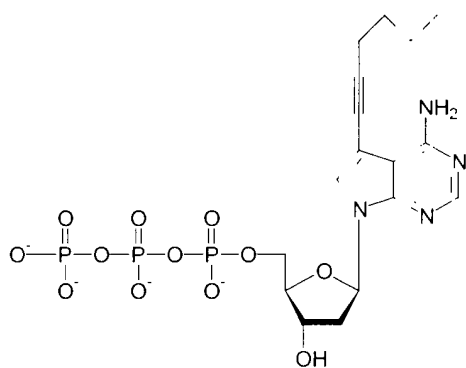
10/019654



Rosamin-dCTP

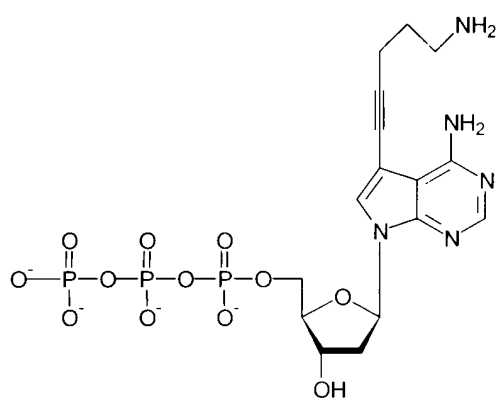


Dig-28-dCTP

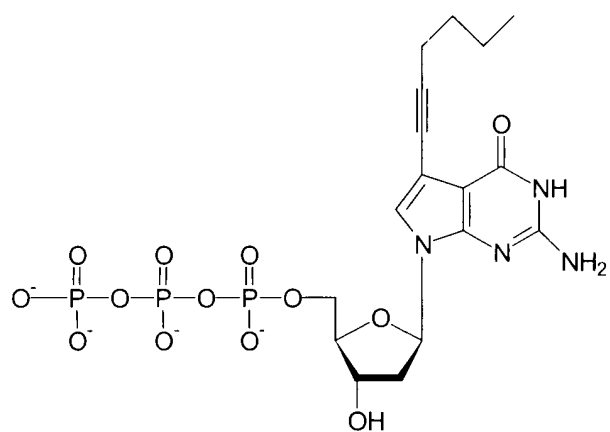


7-Hexinyl-7-desaza-dATP (Hex<sup>7</sup>c<sup>7</sup>Ad)

Fig. 14



7-Aminopentynyl-7-desaza-dATP



7-Hexynyl-7-desaza-dGTP (Hex<sup>7</sup>c<sup>7</sup>G<sub>d</sub>)

Fig. 15

10/019350

List of nucleoside derivatives

d-adenosine and adenosine-analogue derivatives

7-deaza-dATP

Digoxigenin-16-dATP

Fluorescein-15-dATP

Infrared<sub>770</sub>-9-dATP

7-aminopentynyl-7-deaza-dATP

N<sup>1</sup>-[(acetamido)-3,6-dioxa-octylamino]-2'-deoxy-formycin A-triphosphate

biotin-[N<sup>1</sup>-(amidooctyl-3,6-dioxa-amidoacetyl)]-2'-deoxy-formycin A-triphosphate

d-formycin A-triphosphate

N<sup>1</sup>-methyl-formycin-A-triphosphate

7-hexinyl-7-deaza-dATP

Digoxigenin-(7-aminohexinyl-7-deaza)-dATP

TMR-(7-aminohexinyl-7-deaza)-dATP

RhodaminGreen-(7-aminohexinyl-7-deaza)-dATP

d-cytidine- and cytidinanalogue derivatives

Cy5-dCTP

Digoxigenin-dCTP

ψ-iso-dCTP

Rosamin-dCTP

d-guanosine analogue derivatives

8-Aza-dGTP

8-Aza-7-bromo-7-deaza-dGTP

7-aminopentynyl-7-deaza-dGTP

Cy5-(7-aminobutynyl-7-deaza)-dGTP

7-hexinyl-7-deaza-dGTP

Fluorescein-(7-aminohexinyl-7-deaza)-dGTP

RhodaminGreen-(7-aminobutynyl-7-deaza)-dGTP

d-uridine and uridine analogue derivatives

Aminoallyl-dUTP

AMCA-6-dUTP

Biotin-16-dUTP

Bodipy-dUTP

Carboranyl-dUTP

Cy5-dUTP (with 10, 17, 24 and 38 atoms linker length)

DIG-11-dUTP

Fluorescein-12-dUTP

JF9-dUTP

IRD40-dUTP

JA53-dUTP

JA133-dUTP

JA218-dUTP

MR121-dUTP (with 8, 13 and 24 atoms linker length)

RN65-dUTP

RhodaminGreen-dUTP (without 5 and with 12 atoms spacer length)

ψ-dUTP

Biotin-N<sup>1</sup>-ψ-dUTP

TMR-dUTP

Estradiol-dUTP

Other derivatives

[Imidazo-(2-amino-s-Triazin-4-on)]-2'-deoxyribosid-triphosphate

dITP

Chinazolin-2'-deoxyribosid-triphosphate